

# PATENT SPECIFICATION

836,694

NO DRAWINGS.

Inventors:—IAN DOUGLAS MORTON, PHILIP AKROYD and CHARLES GERARD MAY.



Date of filing Complete Specification : April 4, 1956.

Application Date : April 7, 1955. No. 10328/55.

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Index at Acceptance :—Class 49, B(1L : 6A).

International Classification :—A23l.

## COMPLETE SPECIFICATION.

### Flavouring Substances and their Preparation.

#### ERRATA

#### SPECIFICATION NO. 836,694

Page 1, line 59, after "α" insert "and"

Page 1, line 74, for "Cured" read "Crude"

Page 3, line 43, for "L-Cysteine" read "L-cysteine"

Page 3, line 65, for "6.6" read "6.6-6.8"

{ Page 2, lines 63 and 67 and  
Page 3, lines 19, 53, 65 and 82 and  
Page 4, line 27 and  
Page 5, lines 52, 53 and 56 for "pH" read "pH"

Page 4, line 1, for "a" read "α"

Page 5, line 116, for "0.17" read "0.170"

Page 6, line 37, for "gram" read "grams"

THE PATENT OFFICE,

4th July, 1960

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alkaline at the end of the reaction.

- The monosaccharide is preferably a pentose, such as ribose, arabinose, or xylose. Pentoses are generally preferred to hexoses from the standpoint that pentoses give rise to flavours and aromas resembling closely those of cooked meats whereas if a hexose, such as glucose, is used in the absence of pentoses, a substance having a pleasant savoury smell and taste, rather than that of meat, is produced unless heating is continued for a longer time. It may be advantageous to use a mixture of monosaccharides which may contain either pentoses or hexoses or
- also be used. Both racemic and naturally occurring forms of the amino acids can be used. It has been found that fish protein is a suitable starting material for obtaining a mixture of amino acids for the reaction, provided that cysteine is added. Cured fish protein, such as cod flesh, may be used, but preferably, the fish should first be defloured, for instance, by the process described in Specification No. 784,905. The fish protein is then hydrolysed, for instance, by means of hydrochloric acid or caustic soda, and substantially neutralised. The sub-

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International Classification :—A231.

## COMPLETE SPECIFICATION.

### Flavouring Substances and their Preparation.

We, UNILEVER LIMITED, a Company registered under the Laws of Great Britain, of Port Sunlight, in the County of Chester, England, do hereby declare the invention, 5 for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to artificial 10 flavouring substances and to their preparation.

It has been found that flavouring substances capable of imparting to foodstuffs a savoury smell and taste, particularly resembling that of cooked meat, may be obtained by the reaction of a monosaccharide with an amino reagent comprising cysteine in the presence of water and at an elevated temperature, the reaction mixture being not alkaline at the end of the reaction. 15

The present invention provides, therefore, a process for making a flavouring substance which comprises reacting a pentose or hexose monosaccharide with cysteine in the presence 20 of a large excess of water at an elevated temperature, the reaction mixture being not alkaline at the end of the reaction.

The monosaccharide is preferably a pentose, such as ribose, arabinose, or xylose. 25 Pentoses are generally preferred to hexoses from the standpoint that pentoses give rise to flavours and aromas resembling closely those of cooked meats whereas if a hexose, such as glucose, is used in the absence of pentoses, a substance having a pleasant savoury smell and taste, rather than that of meat, is produced unless heating is continued for a longer time. It may be advantageous to use a mixture of monosaccharides which 30 may contain either pentoses or hexoses or

both. Best results are generally obtained if the monosaccharide, used alone or in admixture with other pentose or hexose monosaccharides, is ribose.

Di-, tri-, or polysaccharides or simple derivatives of mono-, di- or polysaccharides which yield the required monosaccharide under the conditions of the reaction may also be used. As suitable monosaccharide derivatives there may be mentioned monoacetone glucose and methyl 45 riboside.

Flavouring substances according to the invention may be obtained by using cysteine as the amino reagent. More full-bodied 50 flavours are generally obtained by using cysteine in admixture with one or more, preferably three or more, other amino acids, such as glutamic acid, glycine,  $\alpha$ - $\beta$ -alanine, threonine, histidine, lysine, leucine, iso-leucine, serine, valine or cystine. It is not essential to use the individual amino acids. Simple derivatives, such as salts, esters or hydroxy compounds, or di-, tri-, or higher peptides, such as glutathione, or proteins, 55 which give rise to the requisite amino acids under the conditions of the reaction, may also be used. Both racemic and naturally occurring forms of the amino acids can be used.

It has been found that fish protein is a suitable starting material for obtaining a mixture of amino acids for the reaction, provided that cysteine is added. Cured fish protein, such as cod flesh, may be used, but preferably, the fish should first be defloured, for instance, by the process described in Specification No. 784,905. The fish protein is then hydrolysed, for instance, by means of hydrochloric acid or caustic 60 soda, and substantially neutralised. The sub- 70

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- stantially neutralised hydrolysate may be used, as such, as the amino reagent, provided cysteine is added. The hydrolysate must be treated, for instance with charcoal, 5 in order to remove phenylalanine and methionine, whilst cysteine or a source thereof must be added before using the mixture. The presence of substantial amounts of phenylalanine or of methionine in mixtures 10 tend to give rise to undesirable floral or potato-like aromas and non meat-like flavours. Small amounts of other aromatic amino acids such as tyrosine and tryptophan may not be objectionable but large amounts 15 should preferably be avoided. Other protein hydrolysates which have been suitably treated to remove phenylalanine and methionine such as groundnut hydrolysate or casein hydrolysate or mixtures of these 20 or hydrolysed keratin may also be used as the amino reagent, provided cysteine is added.
- Factors which may affect the nature and quality of the flavour produced include the 25 nature and relative amounts of the monosaccharide and amino reagents used, the amount of water present and the time and temperature of heating. Thus, to obtain a product having a flavour resembling that of beef the total amino reagent should preferably contain about 1 to 3 times, by weight, of the total weight of cysteine, of amino acids or derivatives which will give rise to amino acids additional to cysteine or of 30 derivatives which will give rise to such amino acids during the reaction. The use of ribose and cysteine in the absence of other amino acids tends to give a flavour akin to that of pork.
- As little as 0.04 mole of monosaccharide per 1 mole of amino reagent may give a satisfactory flavouring substance. By "amino reagent" is meant the total of amino acids and derivatives thereof present, inclusive of 40 cysteine. Amounts of monosaccharide up to 0.8 mole per 1 mole of amino reagent may be used but a greater amount of monosaccharide may be objectionable as it tends to give rise to excessive sweetness of flavour.
- Generally, it is preferred to use from 0.1 to 0.5 mole of monosaccharide per 1 mole of amino reagent.
- Preferred flavouring substances having an 50 aroma and taste akin to cooked meat are obtained when the ratio of cysteine to monosaccharide is between 0.4:1 and 2:1 by weight.
- The amount of water present should, in practice, be at least 5, generally 15 to 25, 55 times the amount, by weight of the total monosaccharide reagent. Amounts of up to 1500 times may, however, be used.
- The pH at which the reaction is carried 60 out is not critical although the mixture at the end of the reaction must not be alkaline.
- It is preferred to carry out the reaction at a pH of between 3 and 6.
- The reaction mixture may be in the form of a solution or in the form of a slurry or sludgy mass, depending on the concentration of reagents and on the conditions of heating. The saccharide may be brought into solution before heating is begun in order to reduce any danger of charring. Stirring of the mixed reagents may be advisable during heating to 70 reduce the danger of local overheating. Vigorous stirring during heating may reduce the time required for completion of the reaction.
- The reaction may be carried out by heating the mixture under reflux. In many cases the mixture is maintained at its boiling point for from  $\frac{1}{2}$  to 4 hours, generally at least for 1 hour. Similar results may be achieved by heating for a longer period at a lower temperature, for instance 70° C., when 24 to 30 hours heating may be required. When using hexoses, it is preferred to heat the mixture 75 under reflux for about 24 hours.
- The process may also be carried out under reduced pressure or under increased pressure when lower or higher temperatures will be necessary to maintain the mixture at or near its boiling point. A reflux condenser is normally fitted to the reaction vessel 80 whilst the reaction is in progress. Conditions of heating should be such as to avoid substantial caramelisation of the mixture.
- The reaction may also be carried out by adding the reagents to a food product in 90 which it is desired to incorporate a savoury or meat-like flavour, such as a protein, and heating the product to effect reaction, for instance by autoclaving in a sealed can. When carrying out the reaction by adding 95 the reagents to a food product it may be necessary to add a small amount of water, but generally such products will already contain sufficient water to enable the reaction to proceed.
- The reaction products may be used as such, or they may be converted to freeze dried powders. Such powders may tend to be hygroscopic.
- Flavouring substances according to the invention may be incorporated in a wide range 100 of meat or meat-like products. They may, for example, be incorporated either as solutions or powders, as is convenient, in protein gels, in luncheon meats, in dry soup mixes 105 and in meat spreads.
- It is preferred to let the flavouring substance age for about a week after its preparation, when optimum flavour is generally developed. The flavouring substance may 110 tend to lose quality after about a month from its preparation. In the absence of air and light the substance or products in which it has been incorporated tend to retain their 115 quality of flavour longer. Freeze-dried 120

powders generally retain quality of flavour for a longer period than solutions.

The following examples illustrate the invention:—

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#### EXAMPLE 1.

The following substances were mixed by stirring in a reaction vessel:—

	Water	360 grams
10	D-Ribose	15 "
	D-Glucose	18 "
	$\beta$ -Alanine	7 "
	L-Cysteine	10 "
	L-Glutamic acid	15 "
	Glycine	5 "

- 15 The vessel was then heated under reflux for 2 hours by means of an oil bath at 130° C. After cooling the product to room temperature and keeping in a closed vessel for 2 days, it was then brought to a pH of 6.6—6.8 by addition of alkali. This brown-coloured solution had a taste and aroma resembling cooked beef; a portion was dried by freeze-drying and the flavouring solution obtained later by reconstituting the powder with water.

#### EXAMPLE 2.

The following substances were mixed and then treated in exactly the same manner as detailed in Example 1:—

30	Water	100 grams
	D-Ribose	5 "
	Glutathione	
	( $\gamma$ -glutamylcysteinylglycine)	10 "

- 35 The product, an orange-coloured, clear solution, had a taste and aroma resembling that of cooked beef.

#### EXAMPLE 3.

- 40 The following substances were mixed and then treated in exactly the same manner as detailed in Example 1:—

Water	300 grams
D-Ribose	3 "
L-Cysteine	9 "

- 45 The product, a pale yellow coloured solution, had a taste and aroma resembling that of cooked pork.

#### EXAMPLE 4.

- Crude cod fish flesh (30 grams) was hydrolysed by refluxing with 6N-hydrochloric acid (200 grams) for 8 hours, then the acid was substantially distilled off at reduced pressure and the residual hydrolysate filtered and brought to pH=6.7 with caustic soda. The dark coloured solution of low molecular weight amino compounds was then passed through a short column (3" x 2") of activated

charcoal, giving a water-white solution. This solution (300 ml., containing 4.2 mg. of Kjeldahl nitrogen/ml.) was then heated to boiling for 3 hours after adding D-ribose (5 g.) and L-cysteine (3 g.), a reflux condenser being fitted to the vessel. After cooling the product to room temperature and keeping it for several days to "age" and mellow the flavour, it was adjusted to pH=6.6 by addition of alkali. The deep-orange coloured solution had a strong taste and aroma resembling cooked beef.

Further experiments were carried out using defloured soya bean protein (30 g.) in place of the crude cod fish flesh, and defloured cod fish flesh (30 g.) in place of the crude cod flesh.

#### EXAMPLE 5.

Commercially available charcoal-treated hydrolysed (casein plus groundnut protein) powder (4 g.) and L-cysteine (2 g.) and D-glucose (1 g.) and D-xylene (1 g.) and water (100 g.) in a vessel fitted with a reflux condenser were heated to boiling for 3 hours. After cooling the product to room temperature, it was brought to pH=6.7 by addition of alkali. The brown-coloured solution had an aroma and taste resembling cooked meat.

#### EXAMPLE 6.

Cod flesh solution prepared by hydrolysis followed by charcoal treatment, as described in Example 4, (50 ml.) and D-ribose (0.8 g.) and L-cysteine (0.6 g.) were added to a bland protein paste prepared from defloured groundnut protein (140 g.) and water (370 g.) in a can. The can was then sealed and autoclaved for 1½ hours at 10 pounds per square inch steam pressure. The can was opened after 6 weeks storing and the contents had a beef-like flavour.

This example was repeated using a defloured soya bean protein paste in place of groundnut protein paste and a similar tasting product was obtained.

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#### WHAT WE CLAIM IS:—

1. A process for making a flavouring substance which comprises reacting a pentose or hexose monosaccharide with cysteine in the presence of a large excess of water at an elevated temperature, the reaction mixture being not alkaline at the end of the reaction.

2. A process according to Claim 1 in which at least one additional amino acid, other than phenylalanine and methionine, is present.

3. A process according to Claim 2 in which at least three additional amino acids, other than phenylalanine and methionine, are present.

4. A process according to Claim 2 or Claim 3 in which the additional amino acid or acids is or are chosen from glutamic acid,

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- glycine,  $\alpha$  and  $\beta$ -alanine, threonine, histidine, lysine, leucine, *iso*-leucine, serine and valine.
5. A process according to any preceding claim in which heating is carried out for from  $\frac{1}{4}$  to 4 hours under reflux.
6. A process according to any preceding claim in which the amount of water is at least 5 times, by weight, the amount of monosaccharide present.
10. 7. A process according to Claim 6 in which the amount of water present is from 15 to 25 times, by weight, the amount of monosaccharide present.
8. A process according to any preceding claim in which the molar ratio of monosaccharide to amino reagent is between 0.04:1 and 0.8:1 by weight.
9. A process according to Claim 8 in which the molar ratio of monosaccharide to amino reagent is between 0.1:1 and 0.5:1 by weight.
10. A process according to any preceding claim in which the ratio of cysteine to monosaccharide is between 0.4:1 and 2:1 by weight.
11. A process according to any preceding claim which is carried out at a pH between 3 and 6.
12. A process according to any preceding claim in which the amino reagent comprises hydrolysed protein.
13. A process according to Claim 12 in which the hydrolysed protein is keratin.
14. A process according to any preceding
- claim in which the amino reagent comprises a peptide. 35
15. A process according to Claim 14 in which the peptide is glutathione.
16. A process according to any preceding claim in which the monosaccharide is ribose. 40
17. A process of incorporating a meat-like flavour in a food product which comprises adding a pentose or hexose monosaccharide and cysteine to the food product in the presence of water and heating the mixture. 45
18. A process of incorporating a meat-like flavour in a protein which comprises adding a pentose or hexose monosaccharide and cysteine to the protein and heating the mixture in the presence of water. 50
19. A process according to Claim 16 or Claim 17 in which the monosaccharide is ribose. 55
20. A process according to any of Claims 1—13 in which the product is converted to a powder by freeze-drying.
21. Flavouring substances whenever made by a process substantially as described herein. 60
22. Food products which contain a flavouring substance whenever made by a process substantially as described herein.

UNILEVER LIMITED,

R. JONAS,

Agent for the Applicants.

#### PROVISIONAL SPECIFICATION.

#### Flavouring Substances and their Preparation.

- We, UNILEVER LIMITED, a Company registered under the Laws of Great Britain, of Port Sunlight, in the County of Chester, England, do hereby declare this invention to be described in the following statement:—
- The present invention relates to artificial flavouring substances and to their preparation.
- It has been found that flavouring substances capable of imparting to foodstuffs a savoury smell and taste, particularly resembling that of cooked meat, may be obtained by the reaction of a monosaccharide or a simple derivative thereof with an amino acid or a simple derivative thereof, such as a salt, in the presence of water and at an elevated temperature.
- The monosaccharide is preferably a pentose, such as ribose, arabinose, or xylose but may be a hexose, such as glucose, fructose or glucosamine. As a simple monosaccharide derivative there may be mentioned monoacetone glucose. It may be advantageous to use a mixture of monosaccharides which may contain either pentoses or hexoses or both. Best results are generally obtained if the monosaccharide used alone or in admixture with other pentose or hexose monosaccharides is ribose. If a hexose, such as glucose, is used in the absence of pentoses, a substance having a pleasant savoury smell and taste, rather than that of meat, is produced. Di- or polysaccharides which yield the required monosaccharide under the conditions of the reaction may also be used. 90
- Flavouring substances according to the invention may be obtained by using cysteine or cystine or a peptide containing a cysteine or cystine residue as the sole amino reagent. Better results are generally obtained by using cysteine or cystine in admixture with one or more, preferably three or more, other amino acids, such as glutamic acid, glycine, tryptophan, tyrosine,  $\beta$ -alanine, threonine, histidine, lysine, leucine, *iso*-leucine or valine. Di-, tri- or higher peptides, such as glutathione, which comprise the requisite amino acids may also be used in the reaction, although peptides of molecular weight greater than about 600 may be unsuitable. 95
- It has been found that fish protein is a 110
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- suitable starting material for obtaining a mixture of amino acids for the reaction, provided that cysteine or cystine is added. Preferably, the fish should first be de-flavoured, for instance, by the process described in Specification No. 784,905. The fish protein is then hydrolysed, for instance by means of hydrochloric acid, and substantially neutralised. The substantially neutralised hydrolysate may be used, as such, as the amino reagent, provided cysteine or cystine is added. The product is improved by treating the substantially neutralised hydrolysate with charcoal in order to remove certain constituents, such as phenylalanine, and then adding cysteine or cystine or a peptide containing these before using the mixture. The presence of substantial amounts of phenylalanine or of methionine in mixtures may give rise to non meat-like flavours. Other suitably treated protein hydrolysates such as groundnut hydrolysate or casein hydrolysate or mixtures of these may also be used as the amino reagent.
- The nature of the meat flavour may be varied by selection of the reagents. Thus, a product having a flavour resembling that of beef may be produced by the use of certain reagents in the reaction, whilst the use of other reagents will give rise to a product having a flavour akin to that of pork. Incorporation of lactic acid or other acids in the reaction mixture in many cases modifies the flavour of the resulting substance to some extent.
- As little as 0.04 mol. of monosaccharide per 1 mol. of amino reagent may give a satisfactory flavouring substance. Amounts of monosaccharide up to 0.8 mol. per 1 mol. of amino reagent may be used but a greater amount of monosaccharide is generally objectionable as it tends to give rise to excessive sweetness of flavour. Generally, it is preferred to use from 0.1 to 0.5 mol., especially 0.3 mol., of monosaccharide per 1 mol. of amino reagent.
- The amount of water present is usually at least 5, generally 15 to 25, times the amount of the total monosaccharide reagent. Amounts of up to 1500 times may, however, be used.
- The pH at which the reaction is carried out is generally not critical although the pH of the mixture at the end of the reaction should not be alkaline. It is preferred to carry out the reaction at a pH between 3 and 6.
- The reaction mixture may be in the form of a solution or in the form of a slurry or sludgy mass, depending on the concentration of reagents and on the conditions of heating. The saccharide may be brought into solution before heating is begun in order to reduce any danger of charring. Stirring of the mixed reagents may be advisable during heating to reduce the danger of local overheating. Vigorous stirring during heating may reduce the time required for completion of the reaction.
- The reaction may be carried out by heating the mixture at or near the boiling point under reflux. In many cases the mixture is maintained at its boiling point for 1 to 4 hours. Similar results may be achieved by heating for a longer period at a lower temperature, for instance 70° C. The process may also be carried out under reduced pressure or under increased pressure when lower or higher temperatures will be necessary to maintain the mixture at or near its boiling point. A reflux condenser is normally fitted to the reaction vessel whilst the reaction is in progress. Conditions of heating should be such as to avoid substantial caramelisation of the mixture.
- The reaction may also be carried out by adding the anhydrous reagents to a food product in which it is desired to incorporate a meat flavour and heating the product to effect reaction, for instance by autoclaving in a sealed can. When carrying out the reaction by adding the reagents to a food product it may be necessary to add a small amount of water, but generally such products will already contain sufficient water to enable the reaction to proceed.
- It is preferred to let the flavouring substance age for about a week after its preparation, when optimum flavour is generally developed. The flavouring substance may tend to lose quality after about a month from its preparation. In the absence of air and light the substance or products in which it has been incorporated tend to retain their quality of flavour longer. Freeze-dried powders generally retain quality of flavour for a longer period than solutions.
- The following examples illustrate the invention.
- EXAMPLE 1.**
- The following substances were mixed by stirring in a reaction vessel:
- |                       |      |       |
|-----------------------|------|-------|
| D-ribose              | 0.15 | grams |
| β-alanine             | 0.11 | "     |
| L-cysteine or cystine | 0.09 | "     |
| L-glutamic acid       | 0.17 | "     |
| DL-tryptophan         | 0.02 | "     |
| L-tyrosine            | 0.07 | "     |
| Water                 | 3    | ml.   |
- Stirring was continued during the subsequent heating. A reflux condenser was fitted to the reaction vessel and this was then heated in an oil bath maintained at 140° for 4 hours. The reaction vessel was then allowed to cool and the flavouring substance was then decanted.
- EXAMPLE 2.**
- A flavouring substance was obtained,

using the same reaction as in Example 1, by mixing together and heating the following substances.

	D-ribose	0.08 grams
5	D-glucose	0.09 "
	Fructose	0.09 "
	$\beta$ -alanine	0.11 "
	L-cysteine	0.10 "
10	L-glutamic acid	0.17 "
	DL-tryptophan	0.05 "
	Water	3 ml.

2.5 ml. (containing 1.26% nitrogen) of the filtrate were added, with stirring, to 0.16 gram of L-cysteine and 0.10 gram of D-ribose in a reaction vessel. Stirring was continued during subsequent heating. A reflux condenser was fitted to the reaction vessel and this was then heated on an oil bath maintained at 140° C. for 45 minutes. The vessel was then taken off the oil bath, cooled to 70° C. and maintained at that temperature for 16 hours. The reaction mixture was then allowed to cool and the flavouring substance decanted.

EXAMPLE 3.  
A flavouring substance was obtained, using the same reaction conditions as those described in Example 1 except that heating was discontinued after 2 hours, by mixing together and heating the following substances:—

	D-ribose	0.15 grams
20	L-cysteine	0.10 "
	Threonine	0.07 "
	Glycine	0.045 "
	L-tyrosine	0.07 "
25	-DL-histidine	0.09 "
	Water	3 ml.

EXAMPLE 4.  
Cod flesh was defloured as described in our co-pending Application No. 24821/54 (Serial No. 784,905). The defloured flesh was hydrolysed with 6N hydrochloric acid and the hydrolysate solution so obtained was neutralised with sodium hydroxide solution, stirred with activated charcoal and then filtered.

EXAMPLE 5.  
Glutathione 0.10 grams  
D-ribose 0.05 "  
Water 1 ml.

were mixed and heated under reflux for 2 hours at 100° C.

EXAMPLE 6.  
Spray dried groundnut/  
casein hydrolysate powder 0.4 grams  
L-cysteine 0.2 "  
D-glucose 0.1 "  
D-xylose 0.1 "  
Water 4 ml.

were mixed and heated under reflux for 2 hours at 100° C. and then 16 hours at about 70° C.

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